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Intergeneric Bacterial Matings

L. S. BARON, P. GEMSKI, JR., E. M. JOHNSON, AND J. A. WOHLHIETER

Department of Bacterial Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012

INTRODUCTION

Our basic interest in promoting intergeneric matings has stemmed from the desire to understand the mechanisms involved in the pathogenesis and host specificity of the various species in the genus *Salmonella*. At the very outset, it seemed to us totally unconscionable that *Salmonella* species would continue to avoid liaisons with the sexually adept K-12 strain of *Escherichia coli*, and, in turn, with themselves. We therefore decided to do what we could to provide a favorable milieu for such mixed matings. The following summation represents a general description of our efforts. We encountered problems in the areas of integration, segregation, and restriction of the transferred genetic material, which we eventually hope we shall overcome.

CHROMOSOMAL HYBRIDIZATION OF *SALMONELLA* AND *PROTEUS* WITH *ESCHERICHIA COLI*

When transfer of genetic material from an *E. coli* K-12 Hfr strain to a *S. typhimurium* recipient was first achieved (L. S. Baron, W. F. Carey, and W. M. Spilman, Intern. Congr. Microbiol., 7th, Stockholm, 1958, p. 50), the immediately apparent difference from the intrastrain K-12 cross was the reduced frequency with which the *Salmonella* recombinants were recovered. The *S. typhimurium* strain, TM-9, employed in those initial hybridization attempts, appeared sterile with respect to its ability to mate with K-12, as, in fact, are the majority of *Salmonella* strains. However, when a sufficiently large number of K-12 Hfr donor cells were mixed with the TM-9 population, recombinants were detected at a very low frequency (about 10^{-8} per donor cell). Therefore, it was theorized that these recombinants arose from rare mutant recipients in the otherwise sterile population which possessed the ability to accept, retain, and express transferred K-12 genetic determinants (3). Consistent with this view was the isolation from the TM-9 population of the fertile mutant TM-9 S^r-2 prior to a mating (2). Transfer of the lactose-utilization gene complex (*lac*) from the K-12 Hfr strain W1895 to TM-9 S^r-2 was observed at a frequency about 10^4 times greater than that observed with the original population. This frequency, however, remains about 100 to 1,000 times less than is normally observed when the lac^+ gene, a lead marker in Hfr W1895 (*see* Fig. 1), is transferred to an *E. coli* F^- strain.

Since we had been concerned with studies in pathogenesis of typhoid fever, our attention was shifted to an *S. typhosa* strain, designated 643, as a potential *Salmonella* recipient. *S. typhosa* 643 had the immediate advantage of being unable to utilize lactose, arabinose (*ara*), rhamnose (*rha*), xylose (*xyl*), or fucose (*fuc*) as carbon sources, (*see* Table 1), thus providing five naturally occurring selective markers. As subsequent studies were to show (13), *S. typhosa* 643 was, in contrast to *S. typhimurium* TM-9, a homogeneously fertile population in which expression of the transferred lac^+ character of W1895 occurred at a frequency of character of W1895 occurred at a frequency of 10^{-5} per donor cell. Moreover, it was, in retrospect, an ideal strain for the demonstration of a second major difference (besides reduced frequency) between the intergeneric crosses and the intrastrain K-12 mating, namely, the predominant occurrence of unstable diploid hybrids.

When *S. typhosa* 643 hybrids which have received any of the carbohydrate-utilization genes (lac^+, ara^+, rha^+, xyl^+, or fuc^+) from *E. coli* Hfr W1895 are streaked on the differential media Eosin Methylene Blue (EMB) or MacConkey agar containing the carbohydrate in question, expression of the positive *E. coli* allele is readily observed. For instance, with EMB medium, carbohydrate-fermenting hybrids appear as black- or dark-centered colonies, whereas nonfermenting strains (like the *S. typhosa* recipient) produce uncolored colonies. In the majority of the *S. typhosa* hybrids recovered from matings with *E. coli* donors, however, stable integration of the *E. coli* gene segment with the resident *Salmonella* chromosome (producing a haploid hybrid) does

not occur. Instead, partially diploid hybrids are formed in which the *E. coli* exogenote is replicated and maintained, with varying degrees of stability, along with the *Salmonella* chromosome. Thus, when carbohydrate fermentation markers are studied, the phenotypic expression of the diploid condition is easily seen. Diploid clones displaying the positive phenotype, upon restreaking on the EMB medium, will continually segregate some haploid, nonfermenting clones from which the positive *E. coli* allele has been lost.

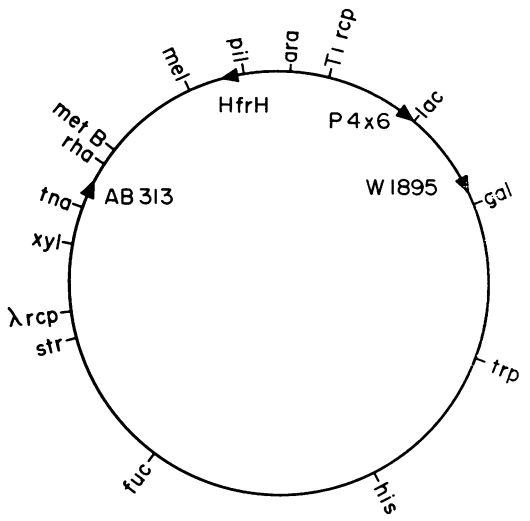

FIG. 1. *Chromosome of Escherichia coli K-12 showing locations of the genetic markers employed. Arrows indicate point of origin and direction of chromosome transfer of the Hfr strains used. Abbreviations are defined in Table 1 except for the following: pil, synthesis of type I pili, T1 rcp, receptor site for phage T1.*

Although the diploid state of *E. coli* × *Salmonella* hybrids is readily demonstrable by the segregation of positive and negative phenotypes, demonstration of integration of the *E. coli* exogenote is not always possible. When the exogenote bears a negative allele, such as, for example, the inability to synthesize methionine (*met*$^-$), and the resident *Salmonella* chromosome carries the positive allele (*met*$^+$), segregation of *met*$^-$ *Salmonella* hybrids is an indication of haploidy and, hence, integration of the *met*$^-$ gene. In the diploid state, it is, of course, the positive (*met*$^+$) *Salmonella* allele which is expressed. Another opportunity for demonstrating integration is provided when the exogenote carries the determinant of streptomycin (*str*) resistance and the *Salmonella* chromosome carries the determinant for sensitivity. Here, the diploid phenotype is streptomycin-sensitive, and streptomycin-resistant segregants from such hybrids must have integrated the *str* locus. However, when the marker in question is a positive carbohydrate-fermentation allele, the question of haploidy or diploidy is, perhaps, never firmly resolved.

We have, in the past, attempted to establish percentages of "stable integration" for the markers *lac*$^+$, *ara*$^+$, *rha*$^+$, *xyl*$^+$, and *fuc*$^+$. We have seen subsequently that markers which appeared to have stably replaced their *Salmonella* alleles on the chromosome, as judged by numerous restreakings on differential media, are, in fact, carried on exogenotes. A case in point is the *E.*

TABLE 1. *Characteristics of parental strains*[a]

Strain	Auxotrophic characters	Carbohydrate utilization							*tna*	*str*
		lac	*ara*	*rha*	*xyl*	*fuc*	*gal*	*mel*		
Escherichia coli K-12										
W1895 (Hfr)	*met*	+	+	+	+	+	+	+	+	S
P4X6 (Hfr)	*met*	+	+	+	+	+	+	+	+	S
H (Hfr)		+	+	+	+	+	+	+	+	S
AB313 (Hfr)	*thr, leu*	−	+	+	+	+	+	+	+	R
Salmonella typhimurium										
TM-9		−	+	+	+	+	+		−	S
TM-9 S^r-2		−	+	+	+	+	+		−	R
S. typhosa										
643	*cys, trp*	−	−	−	−	−	+		−	S
Proteus mirabilis										
WR11	*nic*	−	−				−	−		R
PM-1 F-*lac*	*nic*	+	−				+	−		S

[a] Symbols used: *ara*, arabinose; *cys*, cystine; *fuc*, fucose; *gal*, galactose; *lac*, lactose; *leu*, leucine; *mel*, melibiose; *met*, methionine; *nic*, nicotinic acid; *rha*, rhamnose; *str*, streptomycin; *thr*, threonine; *tna*, tryptophanase (indole production); *trp*, tryptophan; *xyl*, xylose.

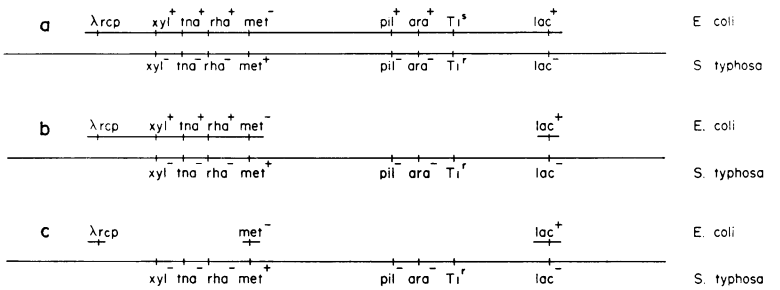

FIG. 2. *Segregation pattern of the E. coli-S. typhosa hybrid X30D. (a) Hybrid X30D showing the presumed extent of E. coli genetic material originally present. (b) Initial segregation of X30D, involving loss of the E. coli pil, ara, T1s region. (c) Further segregation of the hybrid, resulting in loss of the E. coli xyl, tna, and rha genes. No attempt has been made to indicate whether any of the E. coli genes have replaced their Salmonella alleles.*

coli × *S. typhosa* diploid, strain X30D (4). This hybrid is the product of two successive matings between *S. typhosa* strain 643 and *E. coli* K-12 Hfr W1895. In the initial cross, the *Salmonella* hybrid received the selected *lac*$^+$ marker. This hybrid, designated 643L$^+$, appeared to have stably integrated the *lac* marker, and, in the 8 years since its isolation (during which time it has been periodically employed), negative segregants have never been observed. In a backcross of the 643L$^+$ hybrid with W1895 (with selection this time for *xyl*$^+$), a hybrid was recovered which carried, either as exogenotes or endogenotes, the following markers of the *E. coli* parent: *lac*$^+$, *ara*$^+$, *rha*$^+$, *xyl*$^+$, *tna*$^+$ (production of indole from tryptophan), *met*$^-$, *pil*$^+$ (presence of type I pili), and sensitivity to bacteriophage T1 (T1^s). Recently, we have found (E. Penido and L. S. Baron, Bacteriol. Proc., p. 30, 1966) that this hybrid, X30D, also had received the chromosomal determinant of the receptor site for bacteriophage λ (λ *rcp*). The presumed extent of the *E. coli* genetic material contained in X30D is shown in Fig. 2a; no attempt has been made to distinguish possibly integrated haploid genes from nonintegrated exogenotes.

The initial segregation pattern of X30D involved the loss of the region containing the *pil*$^+$, *ara*$^+$, and T1^s markers (Fig. 2b). The *lac*$^+$ marker was retained, and there was no apparent loss of the genes *rha*$^+$, *tna*$^+$, and *xyl*$^+$. Subsequently, it was discovered that *lac*$^-$ segregants could also be obtained (although rarely) from this hybrid, a rather surprising finding since the original 643L$^+$ hybrid, as noted previously, has never been observed to segregate *lac*$^-$ clones. The initial stability of the *rha*$^+$, *tna*$^+$, and *xyl*$^+$ loci, plus the fact that the hybrid was found to be methionine-requiring (indicating integration of *met*$^-$) suggested that the *met* to *xyl* region of the *Salmonella* chromosome had been replaced by the allelic *E. coli* gene segment. Recently, however, this hybrid has been found to segregate clones bearing the negative alleles for the *rha*, *tna*, and *xyl* markers. The presumed extent of *E. coli* material in segregants of this type (which also lack the *E. coli pil*$^+$, *ara*$^+$, and T1^s markers) is shown in Fig. 2c. The *lac*$^+$ marker is retained, although its previously observed loss from the hybrid at the stage shown in Fig. 2b, plus the aforementioned impossibility of demonstration of integration with such markers, precludes any statement regarding its haploid or diploid status. Hybrids of the type shown in Fig. 2c have also been observed recently to segregate the λ *rcp* gene. Thus, of all the *E. coli* genes originally contained in the hybrid X30D, it is evident that only the *met*$^-$ determinant affords the possibility of distinguishing exogenote from endogenote.

Further instances of diploid instability were observed in hybrids obtained from a cross between AB313 and 643. The donor in this mating, an *E. coli* Hfr strain of genotype *xyl*$^+$ *str-r*, transfers its markers as shown in Fig. 1; the 643 recipient is *xyl*$^-$ *str-s*. The cross was performed on minimal xylose media without streptomycin to avoid the lethal effect of the dominant *str-s* gene. In general, the hybrids received only the selective *xyl*$^+$ marker and were unstable. However, the presence of the *str-r* gene was uncovered in one of the hybrids by streaking individual colonies on meat extract-agar plates containing 600 μg of streptomycin per ml. When streaked on this medium, colonies of diploid strain 26D invariably gave rise to a small number of *str-r* colonies, whereas none of the colonies of the sensitive 643 parent behaved in this fashion.

We assume that the *str-r* clones of this hybrid strain must have integrated this region of the *E. coli* chromosome, replacing the lethal effect of the dominant *str-s* locus of the *Salmonella*

chromosome. Furthermore, certain of the *str-r* hybrids also seemed to be stable for the *xyl*$^+$ marker as well, which may indicate that the *xyl*$^+$ marker, as a consequence of integration of the neighboring *str* gene, has also become integrated. Strain 26D was examined also for the presence of the λ *rcp* locus by phage adsorption. The results indicated that this marker was absent despite the presence of the *xyl*$^+$ and *str-r* genes known to bracket the λ *rcp* locus on the *E. coli* chromosome (*see* Fig. 1). As seen previously, portions of the transferred *E. coli* genome initially present can be eliminated at random. Thus, prior segregation of this region may very likely be the explanation for the absence of the λ *rcp* marker in this strain.

It is apparent, therefore, that among the interesting aspects of the conjugal process seen in intergeneric matings between *E. coli* Hfr donors and *Salmonella* recipients are the reduced frequency of marker recovery and the partial diploid nature of hybrids. The low frequency with which *E. coli* genes are recovered in *Salmonella* recipients may involve a number of phenomena. For instance, the presence in a *Salmonella* hybrid of the lead region of an *E. coli* Hfr chromosome increases the frequency with which subsequent *E. coli* genes are recovered upon remating (13). The role of such a resident segment of *E. coli* chromosome, concerned perhaps with early pairing and its effect on marker recovery, still remains to be studied. Likewise, an elucidation of what role restriction-modification (1) plays in the low frequency of intergeneric hybridization must be achieved.

The phenomenon of partial diploidy is equally deserving of further investigation. Although partially diploid hybrids have been recovered from *E. coli* × *E. coli* crosses (5, 14), they are not a common occurrence from such intraspecies conjugations except in cases where *rec*$^-$ recipients are used (15). In contrast, as we have already described, the intergeneric hybridization of *S. typhosa* and *S. typhimurium* with the *E. coli* Hfr chromosome generally results in clones which behave as unstably diploid for the chromosome region transferred. The diploid nature of such hybrids has been interpreted as an expression of poor molecular homology between the parental chromosomes, resulting in inefficient gene pairing and poor gene integration (10).

Even though the existence of partial diploidy in bacteria has been known for a considerable time, many problems remain unsolved. For instance, information about the amount of *E. coli* deoxyribonucleic acid (DNA) present in partially diploid hybrids, about the physical state of diploid pieces, and about the mode of replication, and hence conservation, of the diploid segment is lacking, and questions concerning the interaction of different diploid segments with themselves, as well as with the host chromosome, remain to be clarified. Since the DNA of *E. coli* and the DNA of *Salmonella* have the same overall guanine plus cytosine (GC) base composition, there is no direct method for observing and measuring the amount of *E. coli* DNA in a *Salmonella* diploid.

In a recent approach to the possible solution of some of these problems, we have extended our studies to the chromosomal hybridization of *E. coli* and *Proteus mirabilis*. The differences in overall base composition between *E. coli* (50% GC) and *P. mirabilis* (39% GC) allow a direct physicochemical approach to the study of such intergeneric chromosomal hybridizations. The value of this approach is illustrated by previous studies on the behavior of episomic elements in *Proteus*. Such episomes as the sex factor F, F-merogenotes, colicin factors and multiple drug-resistance factors (RTF) have been conjugally transferred to most of the genera of the *Enterobacteriaceae* [*see* review by Falkow et al. (9)]. The transfer of various F-merogenotes, moreover, from their "natural" host, *E. coli* (50% GC; DNA density, 1.710 g/cc), to hosts like *P. mirabilis* (39% GC; DNA density, 1.698 g/cc) has provided a direct method for examining their DNA base composition. Because of the differences in density between *E. coli* DNA and *P. mirabilis* DNA, the episomal DNA can readily be distinguished from the host chromosomal DNA as a satellite band in CsCl density gradients (11,19). The size of the various episomes can now be determined with this pycnographic procedure. For example, the sizes of a number of F-merogenotes, all consisting of double-stranded native DNA with an average GC content of 50%, have ranged from 2% of the total extracted DNA for the F-*lac* factor (11) to about 11% of the cellular DNA for a large F-merogenote harboring the galactose, biotin, glutamate, and succinate genes of *E. coli* (18). Likewise, the colicine E factor, after transfer to *P. mirabilis*, has been characterized as being about 0.3% of the total cellular DNA and 50% GC in base composition (6). Similar investigations on the genetic material of R factors have also been reported (8, 18).

This fruitful approach to the study of episomes has been recently extended by us to the study of partial diploids. Our investigations on chromosome transfer between *E. coli* Hfr donors and a *P. mirabilis* recipient have revealed that the partial diploids which occur as a consequence of such matings can be studied by a combined genetic and physicochemical approach (12).

By means of plate matings, with the use of either W1895 (Hfr Cavalli) or Hfr H *E. coli* K-12

TABLE 2. *Amount of DNA in the satellite band of Proteus hybrids*

Hybrid strain	Derivation	Hybrid characteristics	DNA in satellite band[a]
WR11		*Proteus* parent	ND
WR13	WR11 × W1895	*lac*$^+$	6
WR24	WR13 × W1895	*lac*$^+$ *ara*$^+$	14
WR14	WR11 × Hfr H	*lac*$^+$	7
WR16	WR13 × Hfr H	*lac*$^+$ *ara*$^+$	20
		lac$^+$ *ara*$^-$ segregant	6
		lac$^-$ *ara*$^-$ segregant	ND
WR18	WR17 × Hfr H	*lac*$^+$ *ara*$^+$ *gal*$^+$	26
WR51	WR13 × P4X6	*lac*$^+$ *mel*$^+$	9
WR31	WR13 × P4X6	*lac*$^+$ *ara*$^+$	15
WR52	WR51 × P4X6	*lac*$^+$ *ara*$^+$ *mel*$^+$	19
WR45	WR13 × P4X6	*lac*$^+$ *ara*$^+$	21
		lac$^-$ *ara*$^+$ segregant	17
WR45	WR45 *lac*$^-$ × W1895	*lac*$^+$ *ara*$^+$	21
		lac$^-$ *ara*$^-$	ND

[a] Expressed as a percentage of the total DNA extracted. ND = none detected.

donors and WR11, a nonswarming *P. mirabilis* recipient, *lac*$^+$ *Proteus* hybrids were recovered at low frequencies. The characteristics of these *lac*$^+$ hybrids are essentially identical (Table 2). In addition to being agglutinated by anti-*Proteus* serum, urease-positive, and sensitive to a virulent *P. mirabilis* phage, these hybrids were unstable for the *lac*$^+$ character. Since no evidence of male properties (i.e., F-pili, male phage sensitivity, and donor ability) was detected, the possibility that these clones were harboring an F-*lac*$^+$ episome could be excluded. We therefore have characterized the *lac*$^+$ *Proteus* hybrids as unstable, heterozygous partial diploids containing an *E. coli* chromosomal segment which encompasses the *lac* region, but which is unassociated with a functional F factor. This interpretation is not unique, because, as we have previously mentioned, partial diploids are common sequellae of intergeneric chromosomal hybridizations. Furthermore, molecular hybridization experiments have indicated only very low levels of molecular homology between *E. coli* and *Proteus* DNA (7; D. J. Brenner and S. Falkow, Bacteriol. Proc., p. 19, 1968).

A physicochemical examination of hybrid DNA provided further evidence of the hybrid character of *lac*$^+$ *Proteus* clones. DNA was extracted by the method of Marmur (16) and centrifuged to equilibrium in a CsCl density gradient, with the use of techniques for observing *E. coli* satellite DNA and parental *Proteus* DNA (11, 19). An example of such an analysis with the WR13 *lac*$^+$ diploid is presented in Fig. 3. The upper curve on the left is a densitometric tracing of the banding of DNA extracted from the WR11 parent strain in a CsCl gradient. The lower curve on the left is a similar tracing of the WR13 hybrid. In addition to the main component, which has a density equivalent to *Proteus* parental DNA, there is a satellite band at a density of 1.710 g/cc, which corresponds to the density of native *E. coli* DNA. This satellite component, about 6% of the total DNA extracted, represents the *lac*$^+$ diploid segment (12). Such estimations are possible if the DNA concentration in the bands is low enough so that the absorption of the light by the DNA and the response of the densitometer will be proportional to the concentration of the DNA in the bands. By measuring the area under the curves, it is possible to estimate the percentage of the total DNA that is in the satellite bands of the diploids. *Lac* diploids similar to WR13 have satellite bands of 6 to 7% of the total DNA. This is larger than the size of the F-*lac* episome and the estimated size of the *lac*$^+$ genes (7). The larger size of this diploid probably indicates that it carries other unselected genes.

By employing a *Proteus lac*$^+$ diploid (WR13) as a recipient in backcrosses with various Hfr donors, other segments of the *E colic* hromosome have been transferred into *P mirabilis*. Partial diploids, hybridized with the *E. coli* chromosomal regions for arabinose, galactose, and melibiose (R. Schmitt, Bacteriol. Proc., p. 122, 1968) utilization, have been recovered at frequencies about 50- to 100-fold greater than that observed for the initial *lac*$^+$ hybridization. Table 2 summarizes the results of CsCl density gradient analyses on representatives of these hybrid classes, and Fig. 3 shows the densitometric tracings of some of them.

We have recently studied some of the nonselected *E. coli* genes that are inherited along with the selected markers in some of these hybrids (P. Gemski, Jr., J. A. Wohlhieter, and L. S.

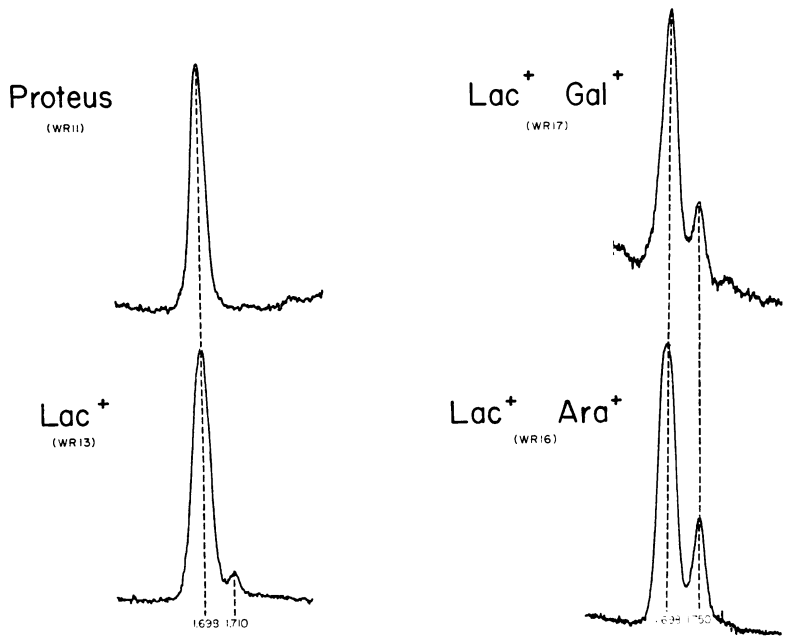

FIG. 3. *Upper curve on the left is a densitometric tracing of the banding pattern of DNA from the parent Proteus strain WR11 in a CsCl density gradient. Lower curve on the left is a tracing of DNA from the Proteus lac+ hybrid (WR13). The main band has the same density as the parent Proteus and, in addition, there is a satellite DNA band at a density of 1.710 g/cc which corresponds to the density of E. coli DNA. The upper tracing on the right is of DNA from the lac+ gal+ hybrid (WR17). The lower tracing on the right is of DNA from the lac+ ara+ hybrid (WR16). The DNA extracted from these hybrids all have additional DNA bands that are the same density as E. coli DNA. From the relative area under the curves, estimates of the percentage of DNA in the satellite bands have been made. (See Table 2.)*

Baron, Bacteriol. Proc., p. 62, 1968). With some of the diploids that contain large satellite DNA bands, it has been feasible to examine as unselected markers the inheritance of *E. coli* type I pili and the receptor for coliphage T1. The locations of the genetic determinants of these characters (*pil* and $T1^s$) on the *E. coli* chromosome are shown in Fig. 1. The results of unselected marker analysis on *lac*+ *ara*+ hybrids are summarized in Table 3. The presence of *E. coli* type I pili was scored by slide agglutination tests, with the use of antiserum prepared against purified type I pili (kindly provided by C. C. Brinton, Jr.). As expected, owing to the proximity of *pil* to the *ara* locus, a proportion of the hybrids had inherited this marker.

Although it has not been possible to demonstrate T1 plaques on *Proteus* diploids, it was found that the presence of the T1 receptor in *Proteus* hybrids could be scored by a test which demonstrates a killing effect. High multiplicities of phage T1, when added to actively growing cultures of some *lac*+ *ara*+ diploids, were found to inhibit the growth of the culture (Fig. 4). Similar experiments with the original parent WR11 or the *lac*+ *Proteus* diploid WR13 showed no effect. Since it was suspected that this phenomenon represented the killing of hybrids which were expressing coliphage T1 receptor, a routine plate test was devised for scoring the presence of the T1 receptor. A high multiplicity of T1 phage was spotted on faint lawns of hybrid clones prepared on meat extract-agar. After approximately 4 hr of incubation at 37 C, clones that had inherited the coliphage T1 receptor were distinctly inhibited in their growth, whereas representative controls remained unaffected. By such a test, a large proportion of the *lac*+ *ara*+ diploids were scored as T1 sensitive.

From a consideration of the combined genetic and physicochemical analysis of these hybrids, it is evident that the more *E. coli* genes the diploid inherits the larger the amount of satellite DNA. Differences have been detected among various hybrids derived from matings with particular Hfr donors. Such variations in satellite size were expected, since we have been able to demonstrate differences in the inheritance of unselected markers (*pil*+ and T1 *rcp*) among diploids.

With the *Proteus* hybrids available at the pres-

TABLE 3. *Inheritance of nonselected markers in lac+-ara+ Proteus hybrids*

Hybrid type	No. tested	*E. coli* type I[a] pili	Coliphage T1[b] receptor
lac+ ara+ (P4X6 × WR13).....	21	20	18
lac+ ara+ (HFR H × WR13).....	24	14	18
lac+ ara+ (W1895 × WR13).....	24	5	22

[a] Agglutination test; antiserum versus purified *E. coli* type I pili.

[b] Tested by spot test.

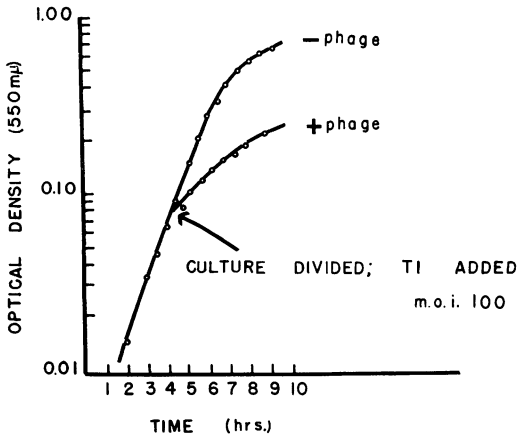

FIG. 4. *Effect of coliphage T1 on the growth of P. mirabilis WR31 lac+ ara+. The culture, growing exponentially at 37 C in aerated minimal arabinose-nicotinic acid medium, was divided, and coliphage T1 was added to one portion. From the inhibition of cell growth by T1, we infer the presence of coliphage T1 receptor. Similar experiments with WR11 and WR13 lac+ showed no effect.*

ent time, we are limited to coliphage receptor genes as well as other surface characters for unselected marker analysis. Some of our difficulties in detecting the presence of such genes in hybrids suggest that problems may exist in the expression of *E. coli* genes and coliphage genes in *Proteus*.

DISCUSSION

From this description of our studies on intergeneric chromosomal hybridizations, we have attempted to highlight the features of the *Salmonella* and *P. mirabilis* systems, the properties of such hybrids, and some of the difficulties encountered in their study. Consistent throughout these observations is the finding that relatively large segments of the *E. coli* chromosome can exist in a diploid state in either *Salmonella* or *P. mirabilis* recipients. Although the failure of *E. coli* genes to replace stably their *Proteus* or *Salmonella* alleles can be rationalized to some degree on the basis of molecular DNA homology differences between the parental systems employed, it is conceivable that other factors, such as the specificity of recombination enzymes (*rec* loci), may also affect intergeneric chromosomal recombination. With the development of knowledge concerning the enzymology of recombination within *E. coli*, it should be possible to determine whether such mechanisms also affect intergeneric gene integration. Likewise, although host restriction and modification probably account in large measure for the low frequency of hybrid recovery, it is probable that other factors are involved as well. Hopefully, the elucidation of such factors may come about as a consequence of continuing studies on the role of restriction-modification in intergeneric hybridizations.

Aside from the reasons for low-frequency hybrid recovery and poor gene integration, one must also consider how a cell is capable of conserving the partial diploid segment. We have alluded previously to a lack of knowledge of the physical state of the *E. coli* chromosomal segments in diploid hybrids. Specifically, we do not know, at present, the nature of the chemical association between exogenote DNA and the resident chromosome or, if such an association does exist, its role in the conservation of diploidy. For instance, it is conceivable that the exogenote DNA is tandemly inserted into the resident chromosome (thus producing a duplicated region within the chromosome) and replicated as part of the continuum of a single DNA duplex. Another possibility is that the exogenote DNA may prove to be an independent replicon, unassociated with the host chromosome. Should this be the case, then questions concerning exogenote replication, as well as the physical form of this DNA, i.e., whether linear (open) or circular (closed), naturally arise.

The hybridization systems described here hopefully can be extended into studies dealing specifically with some of the questions raised. Recent observations by us have revealed that *Salmonella* diploids can be constructed which possess the coliphage λ surface receptor (*mal*A region) and chromosome lysogenization site (*gal* region) among the *E. coli* genes inherited. The effect of restriction-modification and repression on the be-

havior of λ phage and λdg transducing particles within such *Salmonella* hosts is presently under investigation (Baron et al., Bacteriol. Proc., p. 159, 1968). We have noticed that both *S. typhosa* and *P. mirabilis*, after hybridization with some *E. coli* chromosomal regions, act as better recipients of other chromosomal regions upon remating. It is not known, as yet, what function such resident segments have in the expression of this "faithfulness" between diploid recipient and donor, but we conceive of such possibilities as the presence of restriction-modification genes, replication genes, or, for that matter, the importance of early pairing of the donor chromosome with the homologous resident segment of the recipient.

Our investigations on the physicochemical separation of *E. coli* chromosomal segments from *Proteus* hybrids are an extension of earlier findings by us and others (6–8, 11, 18, 19) in separating *E. coli* episomal DNA from *Proteus*. Since this approach has proven useful in demonstrating the circularity of episomal DNA (17), we are hopeful that similar studies on *Proteus* diploids may reveal information about the physical state of diploid segments.

In conclusion, we would like to emphasize that it was not our intention to present an extensive review of intergeneric hybridizations. We chose, rather, to summarize some of our particular interests in the hope that they illustrate some of the broad aspects of this area of investigation. With a better understanding of intergeneric matings, it is conceivable that chromosomal hybridization can be extended to all species and genera of the *Enterobacteriaceae*. Such hybrids would be useful for investigation of virus-bacterial host interactions, host effects on the expression of "foreign" DNA, the genetic basis of virulence, and evolutionary and taxonomic problems.

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